In responding to this rejection, Applicants refer to the remarks presented in the November 12, 1999 Amendment and Response and present the following additional remarks.

The presently claimed invention provides a method for detecting a specific living target cell in a cell suspension of a mixed cell population, in a fluid system containing a mixed cell population, or in a single cell suspension prepared from solid tissue. The detection of a specific living target cell as required by the presently claimed invention requires a high degree of specificity that cannot be achieved by any of the cited references, alone or in combination. None of the references, alone or in combination, teaches or suggests the present invention.

Widder discloses, for example, at the last line of page 11, that the specificity of the *Widder* method is greater than 10 percent. However, the presently claimed invention requires a sensitivity of one percent or less. Such a level of sensitivity cannot be achieved by Widder. The presently claimed invention also provides a method for detecting a specific living target cell. As stated in the November 12, 1999 Amendment and Response, the combination of the teachings of *Widder* and *Connely* would result in detection of killed cells. However, in view of the above amendment, the presently claimed invention is directed to the detection of living cells. Living cells can be used to isolate for example RNA which cannot be done from the dead cells of Widder and Connelly. Withdrawal of the rejection is respectfully requested.

Applicants address the specific comments of the Examiner below.

(a) The Examiner deemed unpersuasive Applicant's argument that *Widder* et al. fail to teach detection of individual target cells as claimed in the present invention. Yet, the Examiner further states that "*Widder* teaches the detection of a population of target cells" (Emphasis added). The presently claimed invention is directed to selection of a limited number of single cells, which is not at all mentioned in *Widder*, because this is not possible with Widder's method.

(b) The Examiner also deemed unpersuasive the argument that the use of Protein A on the microsphere of *Widder* will cause the microspheres to adhere to non-target cells and target cells alike, which results in an unacceptable reduction in specificity. The Examiner stated that a side-by-side comparison would be required to support any assertion that the method of *Widder* is less sensitive than the instantly claimed method. Plasma cells and B-cells contain molecules that bind Protein A. Applicants respectfully assert that if the *Widder* method were used to detect a target cell lacking a molecule capable of binding Protein A in any mixed cell population in which

plasma and B-cells are present, a drastic reduction is specificity would result due to binding of plasma and B-cells to the microspheres of *Widder*. Applicants also respectfully assert that it should be obvious that a lack of specificity would result and that a side-by-side comparison should not be necessary.

(c) The Examiner also deemed unpersuasive the assertion that the method of *Widder* cannot achieve the level of sensitivity required by the instantly claimed invention. The Examiner stated that experimental evidence would have to be provided to support such an assertion. Applicants assert that evidence of the improved sensitivity of the presently claimed method over that of *Widder* can be found by comparing the disclosures of *Widder* and the instant application. *Widder* discloses, for example, at the last line of page 11, that the specificity of the *Widder* method is greater than 10 percent. However, a sensitivity of at least three to six orders of magnitude greater than that disclosed by *Widder* is a minimum sensitivity acceptable for the present method (see, for example, page 8, lines 16-20 an page 21, lines 13-15). As an additional example of the level of sensitivity achievable by the currently claimed method, Applicants submit herewith a copy of Forus et al., J Clin. Pathol: Mol. Pathol., 1999, 52:68-74. In this publication, it is shown that the presently claimed method is capable of detecting 10 malignant cells per 10^7 total cells (see results section of Abstract). Clearly, the *Widder* method cannot provide the sensitivity of the presently claimed method.

(d) The Examiner deemed unpersuasive the argument that the method of *Widder* involves nonspecific binding reactions which is unacceptable. In addition, the Examiner stated that the method of *Widder* employs antibodies on the microspheres which are highly specific for the target cells. Applicants cannot confirm that *Widder* employs highly specific antibodies. *Widder* discloses polyclonal antibodies which are not highly specific. Additionally, due to the inhomogeneous location of Protein A on the particle surface in *Widder*, Protein A with free binding sites will be available during incubation. As a result, particle-particle aggregation may occur, and these aggregates can have non-target cells attached. Such a possibility is unacceptable targeting a low number of single cells as in the presently claimed method. Further, as discussed above, because the method of *Widder* detects cells having molecules capable of binding Protein A in addition to target cells, the method of *Widder* clearly would involve non-specific binding if the target cells lacked a molecule capable of binding Protein A and the mixed cell sample

contained cells capable of binding Protein A. Also, the examples of *Widder* include incubating particles and cells at 37°C. At 37°C monocytes can engulf free particles ant thus increase unspecificity.

Applicants also wish to point out that the use of an incubation temperature of 4°C was not obvious at the time of the invention. In fact, it was counter to common knowledge and intuition because the presently claimed invention requires strong binding and it was known that antibody/antigen binding is best at 37°C. In *Widder* incubation at 4°C is performed to minimize capping, not to maximize specific binding. Therefore, it was surprising that binding at 4°C using the presently claimed invention was sufficiently strong.

(e) The Examiner stated that the method of *Widder* shows that it is possible to obtain particle binding exclusively to target cells. Applicants respectfully assert that exclusive binding to target cells is not possible with the crude and coarse method of *Widder*. In fact, as discussed above, *Widder* discloses that only a sensitivity of greater of 10 percent can be obtained. Such a sensitivity certainly cannot result from exclusive binding. Applicants respectfully request the Examiner to indicate where *Widder* teaches or discloses particle that can exclusively bind to target cells.

(f) With respect to *Connelly*, the Examiner stated that the argument that the fixative used in *Connely* results in killed cells is not persuasive because the argument is directed to a limitation that is not being claimed. Applicants wish to re-assert that the fixative used in *Connely* results in killed cells. Additionally, independent claims 17 and 43 recite the limitation "living" cells. Therefore, unlike the presently claimed invention, the combined the teachings of *Widder* and *Connely* would result in the detection of killed cells.

(g) The Examiner stated that statements regarding the use of detergents were contradictory. Applicants respectfully assert that the statements were not contradictory. Applicants fail to see how a statement that detergent does not alter the sensitivity contradicts a statement that the combination of low detergent concentrations and low incubation temperatures gave surprisingly sufficient specificity. Regardless, in view of the above comments, whether such comments are contradictory have no bearing on whether the presently claimed invention is obvious over *Widder* in view of *Connely*.

Widder et al. in view of Kemmneret al. and Terasaki et al.

The Examiner rejected claims 17–33, 38, and 41–43 under 35 U.S.C. 103(a) as allegedly being obvious over *Widder* et al. in view of *Kemmner* et al. and *Terasaki* et al. for the reasons set forth in paper number 10. Applicants traverse this rejection to the extent that it is maintained.

In responding to this rejection, Applicants refer to the remarks presented in the November 12, 1999 Amendment and Response and present the following additional remarks.

The presently claimed invention provides a method for detecting a specific living target cell in a cell suspension of a mixed cell population, in a fluid system containing a mixed cell population, or in a single cell suspension prepared from solid tissue. The detection of a specific living target cell as required by the presently claimed invention requires a high degree of specificity that cannot be achieved by any of the cited references, alone or in combination.

As stated above (see, for example, under item c) *Widder* cannot achieve the level of sensitivity required by the instantly claimed invention. *Kemmner* does not overcome this deficiency.

Kemmner teaches the use beads for enriching a cell population prepared from solid tumor, and the reference uses the bead/cell complex for assessing the effect of enrichment. However, only 96% of the bead-rosetting cells with specific antibody proved to be tumor cells. Beds coated only with an irrelevant antibody bound 5% of the cells in this sample. Of these 5%, 77% are tumor cells. Moreover, of the 34% cells that bound beads coated with an antibody recognizing the human leucocyte common antigen (Dako-LC), as much as 35% turned out to be tumor cells. Clearly, *Kemmner*does not at all achieve the level of sensitivity and specificity required by the instantly claimed invention since leukocyte common antigen is not expressed on tumor cells. These data demonstrate a highly unspecific method which cannot be used to specifically and reliably detect target cells in a mixed cell population. Therefore, *Kemmner* also fails to teach or suggest a method for detecting a specific target cell in a cell suspension.

Terasaki does not overcome the deficiencies of *Widder* in combination with *Kemmner*. *Terasaki* teaches using hybridoma cell lines and does not refer to binding antibodies to cells. Further, *Terasaki* teaches detection of free antigen in the blood, after all cells are removed from the sample. Therefore, *Terasaki* adds nothing to the other references in this case.

The present invention provides a method for detecting a specific target cell in a population of millions of cells. None of the references, alone or in combination, teaches or suggests the method as claimed. *Widder* simply teaches a coarse separation of blood cells which is nonspecific and provides low sensitivity. Moreover, *Kemmner* also suffers from great non-specificity. Finally, the addition of *Terasaki* with the above references does nothing to cure the deficiencies.

Withdrawal of the rejection is respectfully requested.

CONCLUSION

In view of the amendments and remarks presented herein, Applicants respectfully submit that the claims are in condition for allowance. Notification to that effect is earnestly solicited. If prosecution of this case could be facilitated by a telephonic interview, the Examiner is encouraged to call the undersigned.

Respectfully submitted,

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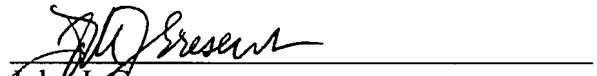

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